Brain tumor imaging using small-angle x-ray scattering tomography

Torben H. Jensen¹, Martin Bech², Oliver Bunk³, Maria Thomsen¹, Andreas Menzel³, Audrey Bouchet^{4,5}, Géraldine Le Duc⁴, Robert Feidenhans'l¹, Franz Pfeiffer²

¹ Niels Bohr Institute, University of Copenhagen, 2100 Copenhagen, Denmark

² Department of Physics, Technical University of Munich, 85748 Garching, Germany

³ Paul Scherrer Institut, 5232 Villigen PSI, Switzerland

⁴ European Synchrotron Radiation Facility, 38043 Grenoble, France

⁵ Grenoble Institute of Neurosciences (Team 7), 38000 Grenoble, France

E-mail: torbenj@fys.ku.dk, franz.pfeiffer@tum.de

Abstract. We demonstrate high-resolution small-angle x-ray scattering computed tomography (SAXS-CT) of soft matter and soft tissue samples. Complete SAXS patterns over extended ranges of momentum transfer are reconstructed spatially resolved from volumes inside an extended sample. Several SAXS standard samples are used to quantitatively validate the method and demonstrate its performance. Further results on biomedical tissue samples (rat brains) are presented that demonstrate the advantages of the method compared to existing biomedical x-ray imaging approaches. Functional areas of the brains as well as tumor morphology are imaged. By providing insights into the structural organization at the nanolevel, SAXS-CT complements and extends results obtainable with standard methods such as x-ray absorption tomography and histology.

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1. Introduction

The properties and functions of many soft matter systems such as soft tissues are often strongly related to the structure at the micro- and nanolevel. Detailed imaging of these structures can provide insight into this correlation. Small-angle x-ray scattering (SAXS) is a widely used method to probe the micro- and nanostructure of noncrystalline materials [Guinier and Glatter(1955), Glatter and Kratky(1982), Narayanan(2009)]. Structural variations in heterogenous samples have been mapped out using a scanning-based set-up and a pencil beam [Fratzl et al.(1997), Rinnerthaler et al.(1999), Paris et al.(2000), Fernandez et al.(2005), Gourrier et al.(2007), Bunk et al.(2009)] . These experiments provide 2D structural information, but often require destructive sample preparation and provide no depth-resolved information about the sample. One way to overcome this limitation is to use x-ray tomography. Standard x-ray absorption computed tomography (CT) has been used for many years for non-invasive 3D studies [Kalender(2006)]. CT provides access to volume-resolved information and requires little sample preparation. While providing excellent spatial resolving power, it has an inherent lack of information about the nanoscale structure of the sample.

It is possible to combine SAXS and CT to small-angle x-ray scattering tomography (SAXS-CT). SAXS-CT has been demonstrated in a few cases with soft matter and biological samples [Harding et al.(1985), Harding et al.(1987), Harding and Kosanetsky(1989), Kleuker et al.(1998), Schroer et al.(2006), Stribeck et al.(2006), Stock et al.(2008)] .

The combination of a scanning-probe imaging technique with tomography requires the acquisition of a vast amount of data. As a consequence, previous studies have been limited to comparatively low spatial resolution. Furthermore, the requirement of fast data acquisition has often compromised SAXS data quality such that only a qualitative analysis was possible, but the rich tools of quantitative SAXS analysis could not be brought to bear. The advent of brighter sources, fast and essentially noise-free detectors, and automated analysis schemes have made possible the efficient acquisition of hundreds of thousands of SAXS patterns that are required to spatially resolve the micro- and nanostructure of extended samples.

In this paper we demonstrate how SAXS-CT can deliver biomedical images that highlight structural (and functional) details of the soft tissue on the nanoscale.

We particularly report results of SAXS-CT experiments performed on a soft matter phantom and on tumors implanted rat brains. Our results on the phantom demonstrate the sensitivity of the method. The obtained information is used to accurately identify and describe the nanostructure of the different samples in the phantom. With the results from the rat brains we demonstrate the use of the structural information for imaging substructures of brain tumors as well as important functional areas of the brain.

2. Materials and methods

2.1. Experimental set-up and principle

Figure 1. Small-angle x-ray scattering tomographic set-up. (a) Using a pencil-beam, scattering patterns are collected by translating the sample through the beam (s), for different orientations (ω) (b)-(c) An example with scattering patterns collected for 4 different projections are shown.

Figure 1a shows the experimental set-up. The idea of the set-up is to use tomographic methods to reconstruct spatially resolved small-angle x-ray scattering curves. Small-angle x-ray scattering is collected in a 1st-generation tomography approach: Using a pencil-beam, 2D SAXS patterns are collected point by point for each horizontal sample translation s , and rotation ω . Figure 1b is a sketch of a sample containing four sample capillaries, with four different beam paths indicated. In Fig. 1c the corresponding azimuthally averaged SAXS curves are shown.

In order to enable tomographic reconstruction of the SAXS signal one of the following demands must be fulfilled: (1) Only the scattering in the q_z direction is reconstructed. (2) The sample scatters isotropically [Schroer et al.(2006)]. In this paper all our samples will be isotropically scattering samples. From this point on the momentum transfer q will hence be treated as a scalar. Scattering from a sample can be described by the differential scattering cross section per unit volume $\frac{d\Sigma}{d\Omega}$:

$$I(s, \omega, q) = I_0 \int_0^L \underbrace{\left[e^{-\int_0^l \mu(\tilde{l}) d\tilde{l}} \right]}_{\text{Abs. before}} \underbrace{\frac{d\Sigma}{d\Omega}(l, q)}_{\text{Scattering}} \underbrace{\left[e^{-\int_l^L \mu(\tilde{l}) d\tilde{l}} \right]}_{\text{Abs. after}} dl \Delta\Omega. \quad (1)$$

The beam path through the sample defined by (s, ω) is parameterized by the variable l over which the integral runs. $I(q)$ is the scattered intensity for a given q -vector, I_0 is the incident intensity, $\mu(l)$ is the local absorption length, and $\Delta\Omega$ is the solid angle covered by the detector element.

$\frac{d\Sigma}{d\Omega}(l, q)$ describes the scattering probability at a certain voxel in the sample. To determine the total scattered intensity we have to take the absorption happening before and after the scattering events into account. Since all scattering occurs at small angles and the samples are limited in size, the scattered photons can be assumed to exit the sample along the same path as the transmitted photons and it is thus possible to separate the absorption and scattering terms. In our set-up the scattering angles are less than 30 and 10 mrad and the sample size less than 3 and 14 mm respectively. This ensures that the path of the scattered photons is approximately equal to the path of the transmitted photons. We define the projected differential scattering cross section $\mathcal{P}(s, \omega, q)$ and rewrite equation (1) as:

$$\begin{aligned}\mathcal{P}(s, \omega, q) &\equiv \frac{I(s, \omega, q)}{I_0 \cdot T(s, \omega) \cdot \Delta\Omega} \\ &= \int_0^L \frac{d\Sigma}{d\Omega}(l, q) dl,\end{aligned}\tag{2}$$

where T is the sample transmission, $T(s, \omega) = e^{-\int_0^L \mu(l) dl}$. Equation (2) has the same form as the line integral of the attenuation coefficient known from absorption tomography [Kak and Slaney(1988)]. It is thus possible to reconstruct $\frac{d\Sigma}{d\Omega}(x, y, q)$ using standard CT reconstruction algorithms for each q . We thus obtain spatially resolved scattering information about the micro- and nano-structures of a sample by reconstructing the local SAXS signal $\frac{d\Sigma}{d\Omega}(q)$ inside an extended sample using standard tomographic methods.

2.2. Sample preparation

The phantom sample was constructed of four capillaries. The capillaries were filled with a colloidal suspension, two types of micelles and silver behenate powder, respectively.

The brain samples were prepared in the following way. The 9L gliosarcoma cell line was originally established by Benda et al. [Benda et al.(1971)]. The 9L gliosarcoma cells were implanted in the brain of male Fisher 344 rats (Charles River, France) as previously described [Regnard et al.(2008)]. Cells were grown with complete medium at 37C. The male Fisher 344 rats (180-280 g, Charles River, L' Arbresle, France) were anesthetized with 4% isoflurane inhalation followed by an intraperitoneal injection of xylazine/ketamine (64.5/5.4 mg/kg). They were placed on a stereotactic head holder (model 900, David Kopf Instruments, Tujunga, USA). At Day 0, 104 cells were suspended into 1 μ l DMEM with antibiotics (1%) then injected using a 1 μ l Hamilton syringe through a burr hole in the right caudate nucleus (9 mm anterior to the ear-bars i.e. at bregma site, 3.5 mm lateral to the midline, 5.5 mm depth from the skull)

[Paxinos (1986)]. The rats were euthanized at different stages of the tumor growth (Day 17 and Day 7 for the two brains respectively). No tumor was visible in the slices from the Day 7 rat. The brains were then sampled and fixed in 10% formalin 10 days minimum prior to any experiment. All operative procedures related to animal care strictly conformed to the Guidelines of the French Government with licenses 380825 and B3818510002 and they were reviewed by the Internal Evaluation Committee for Animal Welfare and Rights of the ESRF.

2.3. Data acquisition and experimental details

The SAXS-CT experiments were carried out at the cSAXS beamline at the Swiss Light Source (SLS) [Bunk et al.(2009)]. Using a monochromatic x-ray beam focused at the sample position, scattering was recorded with a single photon counting PILATUS 2M detector [Henrich et al.(2009)]. The PILATUS 2M detector is essentially noise free and yields excellent statistics, with useful azimuthally averaged photon counts as low as 0.1 photons/pixel.

Two different types of samples were measured. The following experimental parameters were used for the data acquisition for the phantom: x-ray energy 18.6 keV, focus spot size $40 \times 40 \mu\text{m}^2$, sample to detector distance 7212 mm, $30 \mu\text{m}$ step size, 201 steps, 181 angles over 360 degrees. The stepping is done in continuous mode. Exposure time 200 ms for both scattering and absorption measurements. The total acquisition time for the scattering and absorption measurements for one tomographic slice was 4.5 hours. In total 72'000 scattering patterns were collected per slice.

The following experimental parameters were used for the data acquisition for the rat brain samples: x-ray energy 18.58 keV, focus spot size $25 \times 25 \mu\text{m}^2$, sample to detector distance 7117 mm, $25 \mu\text{m}$ step size, 720 steps, 541 angles over 360 degrees. The stepping is done in continuous mode. Exposure time 150 ms for scattering measurements and 10 ms for absorption measurements. The total acquisition time for the scattering and absorption measurements for one tomographic slice approximately 24 hours. In total 390'061 scattering patterns were collected per slice.

Absorption measurements were in both cases conducted with the same set-ups using identical procedures, but with an attenuated beam and no beamstop.

3. Results

To demonstrate the sensitivity of the method a soft matter phantom with several standard SAXS samples was imaged. The phantom was constructed to test the dynamic range as well as the reciprocal space resolution of the set-up. The phantom consisted of four capillaries filled with a colloidal suspension, two types of micelles and silver behenate powder, respectively. Figure 2 displays the experimental results for a single tomographic slice through the phantom. The standard projected absorption sinogram and the corresponding reconstructed absorption lengths are shown in Fig. 2a,b.

Figure 2. Sinograms and reconstructions. SAXS-CT was performed on a phantom consisting of four capillaries. The capillaries were filled with a colloidal suspension (1), two concentrations of micelles (2-3) and silver behenate (4) respectively. (a)-(b) Shows the absorption sinogram and the corresponding reconstruction for a single slice through the sample. (c)-(d) Shows the scattering amplitude sinogram for a specific momentum transfer ($q_1 = 0.049 \text{ nm}^{-1}$), and the corresponding reconstructed differential scattering cross section of the sample. (e)-(f) The same information as (c)-(d), but for a different momentum transfer ($q_2 = 1.0 \text{ nm}^{-1}$). (g) Reconstructed (red) and standard (blue) differential scattering cross sections for the four different capillaries. The reconstructed scattering curves are taken from single voxels (marked in panels b, d and f) and curves from the four capillaries are each offset by two decades for clarity.

Using absorption tomography the four capillaries are clearly localized, but only little information on the micro- and nanostructure of the contents are obtained. Specifically, no information from structures smaller than the voxel size of $30 \times 30 \times 40 \text{ } \mu\text{m}^3$ can thus be resolved. In Fig. 2c-f the projected differential scattering cross section sinograms and the reconstructed differential scattering cross sections for two different q -values are displayed. These reveal information about the samples at the nanoscale. We should note that the momentum transfers we are probing corresponds to real space distances of 4-200 nm. With SAXS-CT we are thus probing length scales that are smaller than the voxel size. Similar reconstructions were made for all recorded values of q , thus reconstructing the full differential scattering cross section for 189×189 different voxels in the slice. The reconstructed differential scattering cross section for four single voxels, each of volume $30 \times 30 \times 40 \text{ } \mu\text{m}^3$ are displayed in Fig. 2g. Their locations are shown in

panels b, d and f. The reconstructed scattering curves of $\frac{d\Sigma}{d\Omega}(x_i, y_i, q)$ (shown in red) are compared to $\frac{d\Sigma}{d\Omega}(q)$ (shown in blue) obtained using the same samples in standard SAXS projection geometry. The later were obtained from projections where only a single component was contributing to the signal, which corresponds to measurements using standard SAXS projection geometry. The projection scattering curves have been normalized to the sample transmission, and sample volume as known from absorption tomography. All curves are reconstructed on an absolute scale. We note that the reconstructed scattering curve of a single voxel inside the sample is identical to the SAXS measured using standard SAXS projection geometry. We thus conclude that we can reconstruct the full SAXS curve associated with a small volume inside our sample.

Since the full scattering curves are known for each voxel, we can conceive many different imaging contrasts signals. In Fig. 2d,f the scattering cross section for a specific q -value is used as imaging signal. A more extensive curve analysis can also be used to generate the image contrast. One way to analyze the curves is to investigate the slope, p , of the scattering curves over a specific q -range by fitting them to a function of the form:

$$\frac{d\Sigma}{d\Omega}(q) = Cq^{-p}. \quad (3)$$

For large values of q this would be equivalent to a Porod-like analysis [Glatter and Kratky(1982)]. One could also make Guinier-like analysis of the curves [Glatter and Kratky(1982)]. Many other more specialized analysis methods can be used to generate image contrast.

The results in Figure 2 demonstrate that a quantitative reconstruction of the local differential scattering cross section is possible with SAXS-CT. The extra structural information on the nanoscale obtained through SAXS-CT now clearly demonstrates its complementarity to tomography based on absorption or phase-contrast. Using the spatially resolved full scattering curves it is possible to identify the content of each of the different capillaries. Capillaries (1), (2) and (3) each contains monodisperse spheres with radius, $r \sim 60$ nm, $r = 110$ nm and $r = 110$ nm respectively. The fourth capillary shows a Bragg peak corresponding to a period of 58.4 nm. We thus obtain important information about the nanostructure of the content of each capillary without having any *a priori* knowledge. We can even see the difference in micelle concentration in capillaries (2) and (3) giving rise to a difference in the differential scattering cross section at small values of q due to static structure factor of the interparticle interaction. The Bragg peak from the material in the fourth capillary is the (0 0 1) peak of the silver behenate crystal powder in the capillary.

Note how the reconstruction is obtained with high q -resolution, demonstrated by the resolving of more than 20 size oscillations in sample number 3. At the same time a high dynamic range is kept, with reconstructed SAXS curves in some cases spanning up to six decades. Even within a specific q -range the high dynamic range is obtained, as seen at the first silver behenate peak where the scattering intensity varies by three decades between different capillaries.

Figure 3. Biological example, rat brain. The three rows, each represent three different tomographic sections of two rat brains. The first column (panels a, c and e) shows the local scattering cross section in the q -range $q \in [0.071 - 0.16] \text{ nm}^{-1}$ for the first row and in the q -range $q \in [0.071 - 0.39] \text{ nm}^{-1}$ for the second and third row. The second column (panels b, d and f) shows the local slope of the scattering curve in the same q -ranges. The scattering curves corresponding to the points marked in this figure are plotted in Fig. 4. The scale bars have a length of 2 mm. All images are displayed with a linear grayscale, with the following limits: a:[5.9-25.3] arb. unit, b:[1.34-2.52], c,e:[8.2-34.2] arb. unit and d,f:[1.80-2.51].

Having introduced and demonstrated the method of SAXS-CT on a set of standard SAXS samples we demonstrate a potential biomedical application by investigating a

Figure 4. Biological example, rat brain. Panels a, b and c shows the reconstructed small-angle scattering curves for 4 selected points from each of the rows in Fig. 3. The panels a, b and c corresponds to row 1, 2 and 3 respectively. The correspondence between the points in Fig. 3 and the scattering curves in this figure is shown by color and numbering. Each scattering curve is offset by a factor of 2 for clarity.

weakly scattering soft tissue sample. Two formalin fixated rat brains were investigated using SAXS-CT. The results are shown in Figure 3. Presented here are three coronal slices from the two different brains. The three different slices are presented in three rows. One brain has two large visible tumors (Fig. 3a,b), a matching slice from the other brain without visible tumor is shown for comparison (Fig. 3c,d). A second slice from the brain without visible tumor is also shown (Fig. 3e,f).

Figure 5. Biological example, rat brain. Panels a and b show the absorption images corresponding to slices c,d and e,f of Fig. 3 respectively. The scale bars have a length of 2 mm. Both images are displayed with a linear grayscale of $[0.83 \ 0.93] \text{ cm}^{-1}$.

A set of scanning absorption images recorded prior to the SAXS measurements with the same setup is shown in Fig. 5 [Bunk et al.(2009)]. The absorption image presented in Fig. 5a(b) corresponds to the slice shown in Fig. 3c,d(e,f). As expected for a soft tissue sample in aqueous environment, only few features within the brain can be distinguished and the overall information content is clearly lower than in the SAXS derived images

[Pfeiffer et al.(2007)].

Panel a in Fig. 3 shows the tomographic reconstructed local differential scattering cross section integrated over $q \in [0.071 - 0.16] \text{ nm}^{-1}$, panels c and e the cross section integrated over $q \in [0.071 - 0.39] \text{ nm}^{-1}$. The intervals are marked by vertical black dashed lines in the plots in Fig. 4. The integrated differential scattering cross section demonstrates sufficient contrast to clearly identify different parts of the brain. The scattering signal provides anatomical maps of the brains. Several functional areas such as the corpus callosum (e.g. Fig. 3f point 4), and the hippocampus (Fig. 3d point 2) are clearly visible, and the tumors (Fig. 3a all points) are clearly delineated.

For each voxel in each slice the full scattering curve has been reconstructed. A few of these scattering curves are shown in Fig. 4. The curves are displayed in Kratky plots ($q, \frac{d\Sigma}{d\Omega} q^2$) and offset by a factor of 2 for clarity. The points from where the scattering curves are taken are marked in column 1 and 2 in the corresponding rows of Fig. 3.

The slope parameter analysis introduced in eq. (3) was performed on all scattering curves of all three slices. The slope parameter, p is displayed in Figure 3 column 2 and was determined over the same q -range as the cross section in column 1.

From the slope parameter we can see increased contrast in several spots. In Fig. 3b, the slope parameter helps to delineate substructures of the tumors that are less visible in the integrated differential scattering cross section image in Fig. 3a. Especially, the necrotic part of the tumors is very well delimited on Fig. 3b (white regions, points 3 and 4). In such a way, using the slope parameter images might help to delineate the active part of the tumor. Also, the edema surrounding the tumors (in the hemisphere bearing the tumor) and the dilation of the ventricle (contralateral hemisphere) are very well defined and limited both on 3a and 3b. From the measurements available it is difficult to determine for a multi component system the exact origin of the variation in slope. There are a number of different possibilities that can result in slope variations. The slope variation may be related to a variation in the local shape at the nanoscale. It may also be related to a variation in relative abundance of scattering structures. Which of these explanations describes best the origin of the variation in the slope of the scattering signal and how this can be linked to tissue changes related to necrosis can only be determined by further studies.

In Fig. 3f the anterior commissure (point 3) is clearly visible when analyzing the slope parameter. Finally in Fig. 3d we can see some of the connections between the internal and external capsule near point 4.

The images in column 2 clearly demonstrate that curve analysis can be used to generate additional image contrast, and hence obtain additional information about the brain and the tumors. In principle all the methods regularly used in small-angle x-ray scattering could also be applied in the analysis of the spatially resolved scattering curves for image contrast and possibly tissue identification.

Although row 1 and 2 show slices through the same region of the brain, only very little of the original structure is left in the tumor invaded brain in row 1. In the tumor invaded brain it is possible to recognize parts of hippocampus and corpus callosum, but

the structure is strongly disturbed by the tumors.

4. Conclusion

In summary, we have demonstrated a high-resolution SAXS-CT over extended q -ranges, which provides information on the local scattering inside an extended sample. This information is obtained by using SAXS in a tomographic approach, thus providing the information in a non-invasive way. Small-angle x-ray scattering curves spanning up to six decades in intensity were reconstructed, with hundreds of thousands of SAXS patterns reconstructed.

We validated the method of SAXS-CT using a phantom comprising several SAXS standards. We thus could demonstrate the range and accuracy to which the scattering curves could be reconstructed. We further showed how the nanostructural information can be used to provide clearly resolved anatomical maps of rat brains. We also demonstrated how the slope of the scattering curves can be used for imaging providing additional contrast for both the functional areas and the tumor. Using the slope delineation of the necrotic regions of the tumor was possible.

The two examples demonstrated how SAXS-CT complements and extends results obtainable with methods such as standard absorption tomography and histology.

We believe that SAXS-CT may be desirable and applicable in many cases. Since the method is non-destructive, samples that cannot be sectioned can now be studied with SAXS. This could include historic and cultural artifacts, and other uniquely valuable samples. It would even be possible to study non fixated tissue, which would be impossible to slice. SAXS-CT could thus also be used to study what transformation tissue undergoes on the molecular level during fixation. For other types of applications, materials science could use SAXS-CT to study sintering of nano- or micro-powder samples.

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